

**REMARKS**

Claim 35 is amended. Claims 1-28 are cancelled. Claims 29-40 are pending.

Support for the amendment can be found on page 24, line 34. Applicants thank the Examiner for the courtesy of a telephonic interview on April 7, 2005.

**Information Disclosure Statement**

Applicants request consideration of the enclosed Information Disclosure Statement, as well as consideration of the Information Disclosure Statement submitted in the previous Response of February 18, 2005.

**Rejection Under 35 U.S.C. § 112, first paragraph- Written Description**

Claims 35, 39, and 40 are rejected by the Examiner due to lack of written description. The Examiner points to a species within the scope of the claim, and argues that the exact species lacks written description. Applicants understand the Examiner's rejection to apply to the exact regulatory element of SEQ ID NO:8 in the wild-type organism. Applicants traverse the rejection on the basis that the rejection is not proper, where the claim language is to a genus rather than the species carved out by the Examiner. Further, applicants point out that regulatory sequences of *Hevea brasiliensis* are known in the art, as presented in applicants' response of February 18, 2005. However, to advance prosecution, applicants amend claim 35 to insert the term "heterologous" as suggested by the Examiner.

**Rejection Under 35 U.S.C. § 112, first paragraph- Enablement**

Claims 29-31 and 34-40 are rejected by the Examiner due to lack of enablement. The Examiner indicates that total alignments as presented previously of the claimed SEQ ID

NO:8 with 3 known acetyl-CoA acetyltransferases indicating both the active site and conserved residues is insufficient, as “no structure/function analysis has been done with the noted sequences,” (Advisory Action, page 4). Applicants respectfully traverse.

Applicants refer once more to Vollack (Vollack et al, Plant. Physiol., 111:1097-1107, 1996, submitted in the IDS of February 18, 2005). Figure 2 of Vollack (page 1100) shows sequence homologies between thiolases at the C-terminal end. The sequences include sequences from man, bacteria, yeast and plants. All of the sequences display the same highly conserved Gly-rich domain in the C-terminal region. These highly conserved amino acid residues can also be found in the claimed sequence (SEQ ID NO:8) of the instant application (see Appendix B, submitted previously) in addition to two highly conserved Cysteine residues, which have been recognized as being important for the thiolase from the slime forming bacteria, *Zooglea ramigera* (gi: 135759). Specifically, Palmer (Journal of Biological Chemistry, vol. 266, no. 13, p. 8369-8375, 1991, previously submitted in the IDS of August 6, 2004) describes the site-directed mutagenesis of the Cys-378 residue to glycine, which provides a mutant enzyme that can no longer catalyze the second half of the enzyme-catalyzed reaction (see Palmer, page 8369, last paragraph). Applicants further note that the crystal structure for a related thiolase was reported in 1994 (see Mathieu et al, Structure, vol. 2, no. 9, p. 797, 1994; cited in the IDS submitted herewith). The crystal structure presented by Mathieu provides detailed structural information on the active site for a thiolase (see Figures 3, 4, and 5). Mathieu provides guidance on the types of substitutions that are likely to be important in thiolases (see particularly page 804, 2<sup>nd</sup> column). Therefore, the alignment and the domains illustrated in Appendix B, combined with site-directed mutagenesis studies and crystal structures known in the art demonstrate that one of ordinary skill in the art could determine which amino acid residues might be modified in SEQ ID

NO:8 while retaining function. Since SEQ ID NO:8 and the radish sequence share 73% identity, one of skill in the art would have appreciated that many variants sharing at least 85% sequence identity to the SEQ ID NO:8 would have been expected to retain acetoacetyl-CoA thiolase activity.

In addition, Applicants submit herewith Appendix C, comparing hydrophilic regions of SEQ ID NO: 8 and the sequence from *R. sativus* (radish, gi: 1542940) according to the method of Kyte and Doolittle (J Mol Biol (1982), 157: 105-132, submitted on the IDS herewith). Applicants note that the plots in Appendix C show almost identical profiles for the two sequences.

With respect to the complementation studies, Applicants refer to Vollack, and note that Vollack was able to isolate a radish (*R. sativus*) acetoacetyl-CoA thiolase using functional complementation of a yeast strain in which the endogenous gene was inactivated. Although the radish and yeast sequence share 51.8% identity, full complementation was achieved, confirming the expectation that acetoacetyl-CoA thiolases from divergent species are functionally similar. In addition, Applicants note that Vollack determined AACT activity using an *in vitro* cell-free assay (see page 1099, 2<sup>nd</sup> column). Further *in vitro* assays are described in Palmer (page 8369, 2<sup>nd</sup> column, 1<sup>st</sup> paragraph).

The Examiner states “what must be enabled is the ability to *make* the claimed invention, not merely *find* the claimed invention via screening procedures,” (Advisory Action, page 4, emphasis in original). Applicants respectfully traverse the Examiner’s apparent requirement to predict from first principles the activity of an enzyme, and request a citation in the art to support the Examiner’s contention that the state of the art requires such a standard.

Applicants assert that the ability to make the claimed invention is fully enabled, particularly where activity is likely to be readily confirmed through art-recognized assays.

Given the above detailed sequence information including identification of the active site by site-directed mutagenesis, a crystal structure of related thiolases, art-recognized assays for activity, several sequence alignments showing conserved residues, and structural analysis via the ability to compare hydrophilicity plots of any changes to SEQ ID NO:8 with both the original SEQ ID NO:8 and with the known radish sequence, one of ordinary skill in the art would be enabled to make and use the invention as claimed without undue experimentation.

### **CONCLUSION**

Claims 32 and 33 are not rejected, but are objected to by the Examiner as depending from rejected claims.

Based on the foregoing amendments and remarks, Applicants respectfully request reconsideration and withdrawal of the rejection of claims and allowance of this application.

### **AUTHORIZATION**

The Commissioner is hereby authorized to charge any additional fees which may be required for consideration of this Amendment to Deposit Account No. 13-4500, Order No. 2119-4268. A DUPLICATE OF THIS DOCUMENT IS ATTACHED.

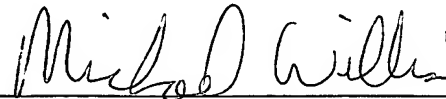
In the event that an extension of time is required, or which may be required in addition to that requested in a petition for an extension of time, the Commissioner is requested to grant a petition for that extension of time which is required to make this response timely and is hereby authorized to charge any fee for such an extension of time or credit any overpayment for

an extension of time to Deposit Account No. 13-4500, Order No. 2119-4268. A DUPLICATE  
OF THIS DOCUMENT IS ATTACHED.

Respectfully submitted,  
MORGAN & FINNEGAN, L.L.P.

Dated: April 19, 2005

By: \_\_\_\_\_



Michael A. Willis

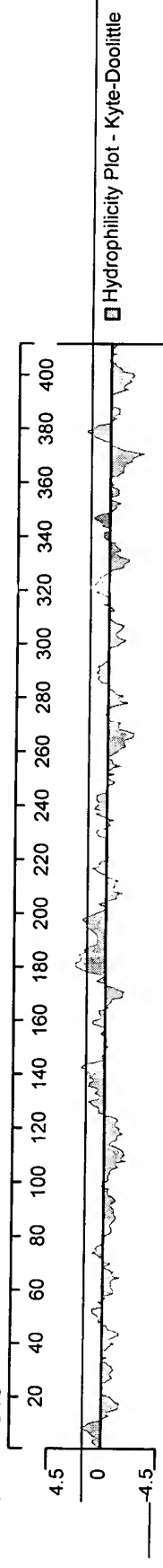
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# Appendix C

SEQ ID NO:8



R.sativus (gi: 1542940)

